MEMORANDUM

Date: October 22, 2009

From: Alan Trounson, PhD
CIRM President

To: Independent Citizen's Oversight Committee

Subject: Extraordinary Petition for Application DR1-01480

Enclosed is a letter from Dr. Gary Steinberg of Stanford University, an applicant for funding under RFA 09-01, CIRM Disease Team Research Awards. This letter was not received at CIRM five working days prior to the October ICOC meeting, but we are forwarding it pursuant to the ICOC Policy Governing Extraordinary Petitions for ICOC Consideration of Applications for Funding.

I have reviewed the petition (referencing reviewer comments and the submitted application as necessary) in consultation with the CIRM scientific staff.

The applicant addresses several concerns raised by reviewers. The petition references information that was not previously provided in the application including findings of efficacy attributed to third group. This information does not have the benefit of expert review by the GWG and does not address the reviewers' primary concern that the efficacy measures were not sufficiently stringent ("cylinder" read-out versus other readout) and their concern that rodent safety efficacy studies alone were not adequate to convince them of the benefit of this therapeutic approach given its risks. Overall, we believe that reviewers carefully considered the notable strengths of this proposal and concluded that despite noted merits, it should not be recommended for funding at this time.

This response provides an overall assessment by CIRM staff, based on our careful review of each of the points raised by the applicant. A point-by-point response would require reference to confidential or proprietary information. CIRM staff is prepared to provide that at the ICOC meeting, should a member so request.

The enclosed letter represents the views of its author(s). CIRM assumes no responsibility for its accuracy.

In addition, a copy of the CIRM Review Summary for this application is provided for reference.

Subject: Extraordinary Petition for DR1-01480: Embryonic-Derived Neural Stem Cells for Treatment of Motor Sequelae following Sub-cortical Stroke

Robert Klein, J.D., Chair of ICOC
Alan Trounson, Ph.D., President of CIRM

Dear Mr. Klein and Dr. Trounson:

Thank you and the CIRM team for the great effort you are expending on behalf of patients. Subsequent to the submission of this application, a third laboratory has further confirmed the therapeutic potential of these cells for treatment of stroke, and on this basis we respectfully submit the attached extraordinary petition for consideration at the ICOC meeting next week.

Yours sincerely,

Gary K. Steinberg, M.D., Ph.D.
Principal Investigator

Gary K. Steinberg, M.D., Ph.D.
Bernard and Ronni Lacroute-William Randolph Hearst Professor of Neurosurgery and the Neurosciences
Director, Stanford Institute for Neuro-Innovation and Translational Neurosciences
Chairman, Department of Neurosurgery
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We thank the reviewers for their highly favorable comments. Subsequent to the submission of this application, a third laboratory has demonstrated behavioral recovery at 8-12 weeks after transplantation of the SD56 cells in a rat MCAo stroke model. Given this third laboratory demonstration of efficacy, we respectfully request our application receive further consideration for funding by the ICOC, to develop this therapy and hopefully meet the critical unmet need of stroke patients with motor deficits. To address the concerns raised by the reviewers, we also provide the responses below.

1. The rodent efficacy models were mild compared to the human condition and did not replicate the underlying atherosclerosis that is the cause of most human stroke. A more physiologically relevant model for efficacy and safety would be additive.
The stroke models in this grant utilize standard and state-of-the-art approaches to modeling stroke in humans. The PIs have extensively reviewed the field of stroke animal modeling and the relevance of each stroke model to the human condition (Carmichael, '05; Carmichael, '08; Horie et al., '08). The models in this grant incorporate the most relevant aspects of the human disease, and do not involve blood vessel atherosclerosis for important scientific reasons. Both stroke models produce strokes in the same arterial territory as most commonly occurs in clinical stroke, the middle cerebral artery. These models produce stroke by occluding the middle cerebral artery or by causing occlusion of the small vessels in the territory of the middle cerebral artery, the two most common stroke subtypes in humans. Rodent models of stroke with underlying atherosclerotic disease do not exist.

Carmichael ST (2005) Rodent models of focal stroke: size, mechanism, and purpose. NeuroRx. 2:396-409.
Carmichael ST (2008) Themes and strategies for studying the biology of stroke recovery in the poststroke epoch. Stroke. 39:1380-8.
Horie N, Maag AL, Hamilton SA, Shichinohe H, Bliss TM, Steinberg GK (2008) Mouse model of focal cerebral ischemia using endothelin-1. J Neurosci Methods. 173:286-90.

2. Behavioral readout is too simple--suggest using a skilled reaching and stepping test that predicts more complex motor restorative potential.
Data is presented for the positive results of the stepping test in Part B, p. 8 (Fig 5). The additional study in the rat MCAo model which forms the basis for this petition also demonstrates behavioral improvement with the SD56 cells on skilled reaching and stepping tests (whisker-paw and catwalk). The proposed studies also include a stepping test and a skilled reaching test (p.13).

3. Not enough preliminary information on the phenotypic fate and migration potential of the NSCs after transplantation in the ischemic brain.
We did not include extensive data on the phenotypic fate and migration potential of the NSCs because this is published (Daadi, '08 & '09). In these publications we quantified the differentiation potential of these cells and their fate and migration after transplantation into stroke. In the grant we propose further studies on fate and migration after transplantation. This work follows on extensive past published experience from the PI and co-PI on cell fate and migration in normal tissue repair and cell transplantation in the brain.

Bliss TM, Kelly S, Shah AK, Foo WC, Kohli P, Stokes C, Sun GH, Ma M, Masel J, Kleppner SR, Schallert T, Palmer T, Steinberg GK (2006). Transplantation of hNT neurons into the ischemic cortex: cell survival and effect on sensorimotor behavior. J Neurosci Res. 83:1004-14.

Daadi MM, Maag AL, Steinberg GK (2008) Adherent self-renewable human embryonic stem cell-derived neural stem cell line: functional engraftment in experimental stroke model. PLoS One. 3:e1644.
Daadi MM, Li Z, Arac A, Grueter BA, Sofilos M, Malenka RC, Wu JC, Steinberg GK (2009) Molecular and magnetic resonance imaging of human embryonic stem cell-derived neural stem cell grafts in ischemic rat brain. Mol Ther. 17:1282-91.
Kelly S, Bliss TM, Shah AK, Sun GH, Ma M, Foo WC, Masel J, Yenari MA, Weissman IL, Uchida N, Palmer T, Steinberg GK (2004) Transplanted human fetal neural stem cells survive, migrate, and differentiate in ischemic rat cerebral cortex. Proc Natl Acad Sci U S A. 101:11839-44.
Ohab JJ, Fleming S, Blesch, A, Carmichael ST (2006) A neurovascular niche for neurogenesis after stroke. J Neurosci. 26:13007-13016.

4. Lack of evidence of in vivo glial cell differentiation.
We have rigorously quantified the phenotype of transplanted cells with this cell line, as noted above. These cells differentiate in vivo into neurons primarily (30.1%), but also into astrocytes (7.1%) and oligodendrocytes (5.7%) (Daadi, '09). However, it is not clear if astrocytic differentiation of transplanted stem cells is important for post-stroke functional recovery. There are no published studies that indicate that astrocytic differentiation promotes behavioral recovery, or correlates with improved tissue repair after stroke. On the other hand, these hES-NPCs do produce functional recovery in two different stroke models. Furthermore, we have shown SD56 cells do secrete various trophic factors in vivo (VEGF, GDNF, IGF, BDNF). With the scientific uncertainties of the role of cell differentiation into astrocytes in stroke repair and recovery, it does not seem valid to *a priori* demand that a candidate cell line for stroke transplantation have a primary astrocytic differentation potential.

Daadi MM, Li Z, Arac A, Grueter BA, Sofilos M, Malenka RC, Wu JC, Steinberg GK (2009) Molecular and magnetic resonance imaging of human embryonic stem cell-derived neural stem cell grafts in ischemic rat brain. Mol Ther. 17:1282-91

5. Further studies regarding the tumorogenic potential of this proposed therapy.
We agree that any cell transplantation approach that utilizes ES -derived cells has a tumorigenic potential. For this reason this grant has included multiple tumorigenicity assays (p.10), including those in normal rats and athymic rats, with survival periods to one year (p.12, p. 15, toxicology sections).

6. hNSCs transplanted into the post-stroke brain will engender adverse inflammatory reactions and exacerbate damage.
This is an experimentally testable idea, and is not borne out from our preliminary and published studies. It will be specifically tested in the studies in this grant (p.13). Transplantation of SD56 cells has not produced an exacerbation of damage or a larger infarct size. In fact, as noted, animals recover significantly better after SD56 transplantation. Also, it should be noted that both in experimental studies in rodents, and in the planned human trial, all subjects will be immunosuppressed. This is necessary because the cells are of course, either xenotransplants (rodents) or allogeneic (humans).

7. Given the potential safety considerations, reviewers questioned the use of this hESC-derived line as opposed to other cell types such mesenchymal stem cells, particularly in the absence of adequate data on mechanism of action.
Mesenchymal stem cells are in clinical trial for stroke. However, these cells may or may not be safer than hES-NPCs. In preclinical studies, most MSCs lodge in peripheral organs and do not

enter the brain (Mäkinen et al., '06; Corerra et al., '07; Schwarting et al., '08). This poses a safety question directly for MSC use. Also, MSCs may be easier to administer for stroke if they are given i.v., but they may not be efficacious. For comparison, i.v. adminstration of MSCs in myocardial infarction did produce a statistically significant improvement in left ventricular ejection fraction, but this was very small (Williams and Keating, '08). Thus, it remains an open question as to whether MSCs will indeed be better than hES-NPCs, and there is no scientifically compelling reason to assume, based on existing published data, that MSCs are necessarily better than hES-NPCs as cell therapy for stroke.

Correa PL, et al (2007) Assessment of intra-arterial injected autologous bone marrow mononuclear cell distribution by radioactive labeling in acute ischemic stroke. Clin Nucl Med. 32:839-41

Mäkinen S, et al (2006) Human umbilical cord blood cells do not improve sensorimotor or cognitive outcome following transient middle cerebral artery occlusion in rats. Brain Res. 1123:207-15.

Schwarting S, et al (2008) Hematopoietic stem cells reduce postischemic inflammation and ameliorate ischemic brain injury. Stroke. 39:2867-75.

Williams BA, Keating A (2008) Cell therapy for age-related disorders: myocardial infarction and stroke--a mini-review. Gerontology. 54:300-11.

8. An earlier decision on the product candidate, cells or cell-matrix combination, as the latter has a much more complex development path.

The decision of cell-matrix vs. cells alone as a transplant strategy is made only 8 months into the grant cycle. This decision is the first no-no go step, and it is placed at a point so close to the grant start date so that the appropriate development path can be taken. The grant has a carefully planned and highly detailed timeline (p. 9) that take into account the development decisions, and factors this very early cell matrix vs. cells alone decision.

9. Immunosuppressive approaches in the preclinical data do not match the proposed clinical immunosuppression regimen.

The pre-clinical studies utilize cyclosporine A and the planned human trial uses tacrolimus. Cyclosporin A was chosen for the rodent studies because of its long track record and substantial validation of dosing and immunosuppressive effect, particularly in stroke or brain transplantation. Both of these drugs immunosuppress through calineurin inhibition. These drugs give comparable immunosuppression (US Multicenter Study Group, '94: English et al., '02; Baiocchi et al., '06) and comparable clinical outcomes (English et al., '02).

Baiocchi L, et al (2006) Cyclosporine A versus tacrolimus monotherapy. Comparison on bile lipids in the first 3 months after liver transplant in humans. Transpl Int. 19:389-95.

The U. S. Multicenter FK506 Liver Study Group. A comparison of tacrolimus (FK 506) and cyclosporine for immunosuppression in liver transplantation. N Engl J Med 1994;331:1110-1115.

English RF, et al (2002) Long-term comparison of tacrolimus- and cyclosporine-induced nephrotoxicity in pediatric heart-transplant recipients. Am J Transplant. 2:769-73.

10. Concern whethert the PI would be able to meet the percent effort commitment given other commitments and whether the fee to a project manager includes other services in the budget item

If awarded this grant, the PI has already made arrangements to reduce his other commitments to devote 30% time to this grant. The budget item for the project manager includes project leadership.

REVIEW REPORT FOR CIRM RFA 09-01: DISEASE TEAM AWARDS I

DR1-01480: Embryonic-Derived Neural Stem Cells for Treatment of Motor Sequelae following Sub-cortical Stroke

Recommendation: Not recommended for funding
First Year Funds Requested: $5,379,111
Final Score:
Total CIRM Funds Requested: $20,000,000

Public Abstract (provided by applicant)
A stroke kills brain cells by interrupting blood flow. The most common "ischemic stroke" is due to blockage in blood flow from a clot or narrowing in an artery. Brain cells deprived of oxygen can die within minutes. The loss of physical and mental functions after stroke is often permanent and includes loss of movement, or motor, control. Stroke is the number one cause of disability, the second leading cause of dementia, and the third leading cause of death in adults. Lack of movement or motor control leads to job loss and withdrawal from pre-stroke community interactions in most patients and institutionalization in up to one-third of stroke victims. The most effective treatment for stroke, thrombolytics or "clot-busters", can be administered only within 4.5 hours of the onset of stroke. This narrow time window severely limits the number of stroke victims that may benefit from this treatment. This proposal develops a new therapy for stroke based on embryonic stem cells. Because our (and others') laboratory research has shown that stem cells can augment the brain's natural repair processes after stroke, these cells widen the stroke treatment opportunity by targeting the restorative or recovery phase (weeks or months after stroke instead of several hours).

Embryonic stem cells can grow in a culture dish, but have the ability to produce any tissue in the body. We have developed a technique that allows us to restrict the potential of embryonic stem cells to producing cell types that are found in the brain, making them "neural stem cells". These are more appropriate for treating stroke and may have lower potential for forming tumors. When these neural stem cells are transplanted into the brains of mice or rats one week after a stroke, the animals are able to regain strength in their limbs. Based on these findings, we propose in this grant to further develop these neural stem cells into a clinical development program for stroke in humans at the end of this grant period.

This proposal develops a multidisciplinary team that will rigorously test the effectiveness of stem cell delivery in several models of stroke, while simultaneously developing processes for the precise manufacture, testing and regulatory approval of a stem cell therapy intended for human use. Each step in this process consists of definite milestones that must be achieved, and provides measurable assessment of progress toward therapy development. To accomplish this task, the team consists of stroke physician/scientists, pharmacologists, toxicologists, experts in FDA regulatory approval and key collaborations with biotechnology firms active in this area. This California-based team has a track record of close interactions and brings prior stroke clinical trial and basic science experience to the proposed translation of a stem cell therapy for stroke.

Statement of Benefit to California (provided by applicant)
The State of California has made a historic investment in harnessing the potential of stem cells for regenerative therapy. While initially focused on developing new stem cell technologies, CIRM has recognized that translational progress from laboratory to clinic must also be fostered, for this is ultimately how Californians will benefit from their investment. Our focus on developing a neuro-restorative therapy for treatment of motor sequelae following sub-cortical stroke contains several benefits to California. The foremost benefit will be the development of a novel form of therapy for a major medical burden: The estimated economic burden for stroke exceeds $56.8 billion per year in the US, with 55% of this amount supporting chronic care of stroke survivors (1). While the stroke incidence markedly increases in the next half-century, death rates from stroke have declined. These statistics translate into an expected large increase in disabled stroke survivors (1) that will have a significant impact on many aspects of life for the average Californian. Stroke is the third greatest cause of death, and a leading cause of disability, among Californians. Compared to the nation, California has slightly above average rates for stroke (2). Treatments that improve repair and recovery in stroke will reduce this clinical burden.

The team that has been recruited for this grant is made of uniquely qualified members, some of whom were involved in the development, manufacturing and regulatory aspects of the first clinical trial for safety of neural stem cells for stroke. Thus not only is the proposed work addressing a need that affects most Californians, it will result in the ability to initiate clinical studies of stem cells for stroke recovery from a consortium of academic and biotechnology groups in California.

1. Carmichael, ST. (2008) Themes and strategies for studying the biology of stroke recovery in the poststroke epoch. Stroke 39(4):1380-8.

2. Reynen DJ, Kamigaki AS, Pheatt N, Chaput LA. The Burden of Cardiovascular Disease in California: A Report of the California Heart Disease and Stroke Prevention Program. Sacramento, CA: California Department of Public Health, 2007.

Review Summary

This application proposes to treat stroke motor deficits using an allogeneic neural stem cell (NSC) line derived from human embryonic stem cells (hESC), delivered alone or in combination with a matrix material into the infracted area in the brain with concomitant immunosuppression. The proposed therapeutic approach is based on the hypothesis that the transplanted cells will stimulate endogenous repair mechanisms and that their survival and the duration of their neurorestorative activities will be enhanced through combination with matrix material. The applicant will conduct preclinical experiments evaluating graft targeting with and without matrix material, optimal timing for transplantation, cell dose, tumorgenicity, and functional recovery using sensorimotor function improvement in rodent models. Additionally, the applicant outlines plans for GMP cell line manufacturing plan, an early pre-IND meeting and other appropriate IND-enabling activities.

Reviewers agreed the scientific rationale for this proposal is solid. There has been a resurgence of interest in treating stroke with cells which may in part reflect that fact that other approaches to stroke therapy have not yielded any new effective drugs. The rationale for transplantation of NSC as a therapeutic approach after neurological injury of any kind is sound. There are numerous examples of promising pre-clinical data in neurological models of stroke, including data from preclinical rodent models of subcortical ischemia where return of motor function occurred after cell delivery. The mechanisms involved remain to be fully elucidated (providing trophic support, promoting angiogenesis, restoring synaptic circuitry, promoting intrinsic cerebral cell repair). Regardless of the precise mechanism ultimately described, NSC show promise as an approach to stroke therapy. Reviewers noted that cell based therapies based on autologous bone marrow cells and allogeneic mesenchymal stem cells are being explored by others as therapy for ischemic stroke.

The significance of this proposal for stroke therapy, if successful, is high. Stroke has the highest annual incidence of any neurological disorder and is a leading cause of disability in adults. There are limited therapeutic options. Thrombolytic agents delivered in the early acute phase of stroke are the only effective pharmaceutical interventions, and most patients do not receive treatment within the limited time when these agents are effective. There is no effective agent that protects when given later. Therefore, any therapy that improves or returns function to these stroke patients will have considerable impact.

Reviewers considered the preliminary data to be supportive of the maturity of the proposed candidate but had some concerns. Reviewers noted that the applicant presents evidence that the proposed cell therapy improves motor function in rodent stroke models, but agreed the rodent efficacy model was mild compared to the human condition. The models also did not replicate the underlying atherosclerosis that is the cause of most human stroke. Additionally, they considered the efficacy readout to be too simple and suggested using a skilled reaching and stepping test that predicts more complex motor restorative potential. Although the preclinical studies suggest the stability of the NSC for up to two months after transplantation, reviewers thought that there was not enough preliminary information on the phenotypic fate and migration potential of the NSC after transplantation in the ischemic brain. Emphasis on these issues is critical in evaluating the safety of these cells as a therapeutic strategy for stroke. One reviewer expressed concern regarding preliminary data on in vivo differentiation of the cells. Whereas

differentiation into neurons was determined, there was the apparent lack of evidence of in vivo glial cell differentiation which produce trophic factors that enhance neurogenesis and synaptogenesis and no mention if cells in the matrix material can differentiate. Reviewers uniformly highlighted the tumorigenicity risk and suggested the need for further studies regarding the tumorogenic potential of this proposed therapy. Several reviewers commented that a more physiologically relevant model for efficacy and safety would be additive. The applicant mentions a potential need to do such model studies, but there is no information on how to execute this. Specifically, there isn't a well established physiologically relevant model of stroke that is easily reproducible. Some reviewers raised a concern about the potential harmful effects of inflammation. This allogeneic therapy will commence two weeks after an acute stroke raising the question of whether administration of the NSC to the brain, especially a brain that has a blood brain barrier disrupted by stroke and is therefore more accessible to subsequent inflammation, will engender adverse inflammatory reactions and exacerbate damage. This necessitates stringent safety studies. Given the potential safety considerations, reviewers questioned the use of this hESC-derived line as opposed to other cell types such mesenchymal stem cells, particularly in the absence of adequate data on mechanism of action.

Reviewers were generally positive about the development plan although they considered it incompletely developed in light of some of the points raised above. All reviewers agreed the pre-pre- IND meeting was an excellent idea and may serve to determine approaches to address some of the safety and model questions. Milestones were generally clear and well-articulated and activities were planned to be performed in parallel. Most reviewers thought the IND could be achieved in four years assuming that FDA does not require studies in a physiologically relevant model. Reviewers encouraged an earlier decision on the product candidate, cells or cell-matrix combination, as the latter has a much more complex development path which could also delay the timeline for IND filing. Reviewers indicated that if this will be a combination product then all the characterization assays, potency, safety tests, etc. will need to be done with the matrix material as well as the cells. Another reviewer expressed concern that the immunosuppressive approaches in the preclinical data do not match the proposed clinical immunosuppression regimen and strongly encouraged preclinical studies with the proposed regimen. Reviewers unanimously agreed that a strength of this proposal is the Principal Investigator (PI) and the very qualified team. The PI and Co-PI have extensive experience in stem cell biology research and in stroke clinical trials. They have put together an excellent leadership team including establishment of an ethics and patient advocacy advisory committee and scientific advisory committee. One reviewer expressed concern as to whether the PI would be able to meet the percent effort commitment given other commitments. Reviewers also were positively impressed with the selection of consultants who had expertise in GMP manufacturing of cell therapy agents, regulatory experience and toxicology. Reviewers thought the budget was adequate, although one reviewer questioned the fee to a project manager and wondered if other services were included in this budget item.

Overall the reviewers agreed this proposal addresses a critical unmet medical need in the therapy for stroke patients. The knowledge and experience of the investigators and the team was highly touted. However the reviewers were not convinced the preclinical models were rigorous enough to provide compelling data to affirm a positive therapeutic effect in patients and questioned the risk benefit given the safety issues of this cell line derived from hESC.

The following scientific Grants Working Group members had a conflict of interest with this application:
Weber, Darrin